

## Aberystwyth University

### *The effect of dietary fat and metabolizable energy supply on milk protein concentration of dairy cows*

Moorby, Jon; Dewhurst, Richard J.; Thomas, Cledwyn; Stuart, Marsden

*Published in:*  
Animal Science

*DOI:*  
[10.1017/S1357729800009735](https://doi.org/10.1017/S1357729800009735)

*Publication date:*  
1998

*Citation for published version (APA):*

Moorby, J., Dewhurst, R. J., Thomas, C., & Stuart, M. (1998). The effect of dietary fat and metabolizable energy supply on milk protein concentration of dairy cows. *Animal Science*, 67(1), 1-8.  
<https://doi.org/10.1017/S1357729800009735>

#### **General rights**

Copyright and moral rights for the publications made accessible in the Aberystwyth Research Portal (the Institutional Repository) are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Aberystwyth Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Aberystwyth Research Portal

#### **Take down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

tel: +44 1970 62 2400  
email: [is@aber.ac.uk](mailto:is@aber.ac.uk)

## The effect of dietary fat and metabolizable energy supply on milk protein concentration of dairy cows

J. M. Moorby<sup>1†</sup>, R. J. Dewhurst<sup>1†</sup>, C. Thomas<sup>1</sup> and S. Marsden<sup>2</sup>

<sup>1</sup>Grassland and Ruminant Science Department, Scottish Agricultural College, Auchincruive, Ayr KA6 5HW

<sup>2</sup>Dalgety Agriculture Ltd, 180 Aztec West, Almondsbury, Bristol BS12 4TH

### Abstract

To investigate the effect of dietary fat and metabolizable energy (ME) on milk protein concentration, an experiment was carried out using 12 multiparous early-lactation Holstein-Friesian dairy cows. Three diets were offered in a complete Latin-square change-over design, based on *ad libitum* access to grass silage. One of three concentrates was offered at a rate of 12 kg/day, each formulated to supply one of two levels of ME (12.1 and 13.6 MJ/kg dry matter (DM)) and one of two levels of fat (31 and a mean of 88 g acid hydrolysis ether extract per kg DM): low energy, high fat (LEHF); low energy, low fat (LELF); and high energy, high fat (HEHF). The concentration of milk protein was significantly higher from animals offered the LELF concentrate (32.5 v. a mean of 31.2 (s.e.d. 0.45) g/kg,  $P < 0.05$ ), because of lower milk yields (31.0 v. a mean of 33.4 (s.e.d. 0.63) kg/day,  $P < 0.05$ ). Animals offered the HEHF concentrate produced the highest yields of milk protein but their milk had the lowest concentrations of fat (32.5, 34.4 and 31.9 g/kg for LEHF, LELF and HEHF respectively; s.e.d. 1.07;  $P < 0.05$  for difference between LELF and HEHF). Silage DM intake was significantly increased by animals offered the LEHF concentrate (9.1, 8.6 and 8.7 (s.e.d. 0.19) kg/day,  $P < 0.05$  for differences between LEHF and the other two concentrates). Urinary purine derivative excretion, used as an index of microbial protein supply, was highest from animals offered the LELF and HEHF concentrates, which both supplied similar amounts of fermentable ME. It is hypothesized that increased *de novo* synthesis of fatty acids on the low fat diet reduced the availability of glucose for lactose synthesis, leading to reduced milk yields and hence increased milk protein concentrations.

**Keywords:** dairy cows, dietary fat, metabolizable energy, milk production, milk protein.

### Introduction

In early lactation, many dairy cows lose condition as energy output in milk exceeds dietary energy intake. With the need to increase the cost efficiency of milk production, fat may be used as a relatively cheap energy source for incorporation into dairy cow lactation rations. However, milk protein concentration has become an important consideration and although an increase in fat consumption by the dairy cow tends to increase milk yields, it also tends to decrease milk protein concentration (DePeters and Cant, 1992).

At high levels of incorporation fat can adversely affect fibre fermentation in the rumen (Coppock and Wilks, 1991). Saponification of oils with calcium or the use of whole oil seeds can reduce this problem by partially 'by-passing' the rumen. In this respect, rumen acetate concentration was increased by increasing the level of rumen protection of supplemental fat (Jenkins and Jenny, 1992) and this was associated with a concomitant increase in milk yield. At the same time, however, milk protein concentration was seen to decrease slightly (Jenkins and Jenny, 1992). Other workers have found a similar effect: increasing fat supplementation resulted in increased milk yields but a decrease in protein concentration (Drackley and Elliot, 1993). Casper and Schingoethe (1989) proposed that this phenomenon is mediated by a reduction in growth hormone release and an indirect reduction of amino acid

† Present address: Animal Science and Microbiology Department, Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth SY23 3EB.

uptake by the mammary gland. Cant *et al.* (1993), on the other hand, postulated that additional dietary fat decreases mammary blood flow, thereby reducing the delivery of nutrients to the mammary gland. Recent attempts to reduce the effect of dietary fat on milk protein concentration by, for example, the use of protein-fat 'bypass' supplements (Holter *et al.*, 1993) or high levels of undegradable protein (Palmquist *et al.*, 1993) have met with only limited success.

Intramammary nutrient partitioning, particularly of energy-yielding substrates such as glucose and acetate, can affect the quantity and quality of milk produced. Fatty acids absorbed from the diet can be incorporated into milk fat unchanged (Banks *et al.*, 1980) and an increase in the output of long-chain fatty acids in milk is associated with a decrease in the *de novo* synthesis of short-chain fatty acids (Faulkner and Pollock, 1989). The extent to which this happens is indicated by the concentration of citric acid in milk (Faulkner and Pollock, 1989), allowing the effects of diet on mammary fatty acid synthesis to be examined and an increased understanding to be gained of the intramammary processes involved in milk production from animals on specific diets.

This experiment was designed to investigate the effect of offering two levels of dietary fat at two concentrations of fermentable metabolizable energy (FME) on milk production and composition in dairy cows. Urinary purine derivative excretion was used as an index of microbial protein supply and milk citric acid concentration as an index of mammary fatty acid synthesis.

## Material and methods

### *Animals and their management*

Twelve multiparous Holstein-Friesian cows, at weeks 8 to 10 of lactation at the start of the experiment, were drawn from the Scottish Agricultural College Auchincruive herd. They were housed in a metabolism unit in individual stalls fitted with de Boer yokes and were milked *in situ* using a vacuum line and bucket units at about 05.30 h and 15.30 h. Milk yields were recorded at each milking by weighing.

### *Experimental design*

The experiment was a complete change-over design based on four 3 × 3 Latin squares. Each experimental period was divided into adaptation and collection periods of 3 weeks and 1 week in length respectively. The mean milk yields from the 7 days prior to the experiment were used to allocate animals to Latin squares, with the three lowest yielding cows assigned to square one, the next three to square two and so on to the three highest yielders in square four.

Within squares, the three treatments were allocated at random to each animal.

The data obtained were analysed statistically using analysis of variance with GENSTAT 5 (Lawes Agricultural Trust, 1990). A blocking structure of period × (square/cow) and a treatment structure of experimental diet were used. For the analysis of urinary purine derivative excretion data, a treatment structure of diet × day × time was used. Because of the non-orthogonal nature of the treatment structure, differences between diets were assessed using a *t* test.

### *Diet formulation and production*

The experimental diets were based on *ad libitum* access to first-cut grass silage. This was supplemented with concentrates offered at a flat rate of 12.0 kg/day which were formulated to provide between them two levels of acid hydrolysis ether extract (AHEE) and two levels of metabolizable energy (ME). Dry-matter (DM) content, crude protein (CP) content, protein degradability and the ratio of starch to digestible crude fibre (a component of the food compounder's formulation matrix) were all formulated to be similar across the three concentrates. The composition of each of the three concentrates, low energy, high fat (LEHF), low energy, low fat (LELF) and high energy, high fat (HEHF) is given in Table 1. The lower ME level was intended to be moderate in terms of the requirements for a dairy cow in post-peak lactation. The concentrates consisted of a relatively low quality carbohydrate energy source plus added fat (mainly

**Table 1** Summary of the ingredient composition of the three experimental concentrates (g/kg fresh weight)

	Concentrate		
	LEHF	LELF	HEHF
Barley		241	253
Wheat		104	140
Wheatfeed	297		
Rice bran	223		
Molassed sugar-beet pulp	117	233	92
High protein maize gluten meal	25		
00-Rapeseed meal	124		200
Sunflower seed meal		179	35
Field beans		175	25
Toasted soya hulls			75
High protein soya-bean meal			23
Fat	10		10
Palm oil	40	6	40
Molasses	100	40	77
Minerals and vitamins	64	22	30

palm oil) (LEHF), a higher quality carbohydrate energy source with very little added fat (LELF) or the LELF energy sources plus the LEHF added fat (HEHF). Therefore, in addition to two contrasting energy levels and two contrasting rates of fat inclusion, the three concentrates offered two contrasting FME levels. The logical fourth concentrate that would have allowed a  $2 \times 2$  factorial investigation would have been one containing high energy and low fat. This was not possible, however, because an increase in ME beyond that achieved on the LELF concentrate was not achievable within the bounds of the other formulation criteria.

#### *Animal feeding*

Cows were offered fresh grass silage *ad libitum* daily at approximately 09.30 h. This was done by offering proportionately about 0.1 more silage than the previous day's intake and by topping up individual animal's food bins during the day if necessary. The concentrate part of the diet was offered in two equal portions of 6.0 kg at each milking.

The silage offered during period 1 was a first-cut grass silage prepared with a formic acid silage additive (Add-F, BP Nutrition (UK) Ltd. Northwich, Cheshire). A new clamp was opened at the end of period 1 and a first-cut grass silage that was prepared using a bacterial inoculant (EcoSyl, ICI Bio Products, Billingham, Cleveland) was offered during periods 2 and 3.

#### *Sample collection and analysis*

During the last 10 days of each experimental period, silage intake was measured by weighing out the silage offered and weighing back the refusals the following morning. Small samples (approx. 200 g) of the silage offered were collected daily and bulked for analysis. Similarly, approximately 200 g of silage refusals were collected from each animal and bulked over the 10 days for DM analysis. Silage samples were frozen immediately after collection and stored at  $-20^{\circ}\text{C}$  until analysed. Concentrate samples were bulked over each experimental period and stored at  $-20^{\circ}\text{C}$  until analysed.

Spot urine samples were taken by vulval stimulation at about 10.30 h and 14.30 h on each of 2 days consecutively at the end of each collection period. The samples were immediately diluted 1 in 20 (75  $\mu\text{l}$  urine in 1.5 ml) with 0.1 mol/l ammonium dihydrogen orthophosphate solution to avoid the precipitation of uric acid from undiluted urine when frozen and thawed. The diluent also contained 0.1 mol/l allopurinol as an internal standard.

The samples were either analysed immediately for creatinine and the purine derivatives uric acid and

allantoin, or were frozen upright and stored at  $-20^{\circ}\text{C}$  until analysed.

Samples of faeces were collected from each cow at the same times as urine samples, taking care to avoid contamination. The samples were frozen immediately and were stored at  $-20^{\circ}\text{C}$  until being dried at  $60^{\circ}\text{C}$  for storage before later analysis for indigestible acid-detergent fibre content (Penning and Johnson, 1983).

Milk yields were recorded daily throughout the experiment. Milk samples were taken at four consecutive milkings, starting with an afternoon milking, and were preserved using Lactab milk preservative tablets (Thompson and Capper Ltd, Runcorn, Cheshire, UK) and storage at  $4^{\circ}\text{C}$ . Samples from the first two milkings of the four were also taken for analysis of milk CP, casein, non-protein nitrogen and urea; subsamples of this were frozen and stored at  $-20^{\circ}\text{C}$  for later analysis of milk minerals.

Methods of analyses of food, faeces, urine and milk were carried out as described by Moorby *et al.* (1996).

## Results

The mean composition of the silages offered during the experiment is presented in Table 2 and the composition of the concentrates offered in Table 3. Silage DM changed between experimental periods

**Table 2** Composition of silage offered throughout the experiment (mean of three samples, each bulked over 10 days; values in g/kg dry matter (DM) unless otherwise stated)

	Mean	s.d.
Dry matter (g/kg)	254	49.6
Organic matter	932	0.6
Crude protein	182	9.5
Metabolizable energy (MJ/kg DM)	11.5	0.06
Rumen degradable protein	155	8.4
Undegradable protein	27	1.2
Neutral-detergent fibre	435	14.2
Acid-detergent fibre	261	9.5
Water-soluble carbohydrates	40	24.3
Ether extract	46	6.4
Acid hydrolysis ether extract	56	6.3
<i>In vitro</i> organic matter digestibility (g/kg OM)	784	4.9
D-value	718	3.8
$\text{NH}_4\text{-N}$ (g/kg total N)	89	30.9
pH	3.7	0.06
Calcium	6.2	1.40
Phosphorus	3.4	0.67
Magnesium	2.5	0.60
Potassium	20.8	2.71
Sodium	4.0	1.29

**Table 3** Composition of the experimental concentrate portions of the diet (values in g/kg dry matter (DM) unless otherwise stated)

	Concentrate		
	LEHF	LELF	HEHF
Dry matter (g/kg)	858	857	860
Organic matter	864	918	913
Crude protein	191	185	182
Metabolizable energy (E3) (MJ/kg DM)	12.1	12.1	13.6
Fermentable metabolizable energy† (MJ/kg DM)	9.1	11.1	10.8
Ether extract	84.5	18.7	77.8
Acid hydrolysis ether extract	92.0	30.6	84.2
Starch	128	238	240
Water-soluble carbohydrates	105	97.4	93.4
Acid detergent fibre	128	139	128
<i>In vitro</i> organic matter digestibility (g/kg OM)	698	811	779
Calcium	16.7	10.5	14.3
Phosphorus	9.6	6.1	7.0
Magnesium	4.6	3.4	3.5
Potassium	17.1	14.4	12.7
Sodium	4.3	3.2	3.1

† Estimated: FME = ME - 0.033 × AHEE.

(202, 259 and 301 g DM per kg for periods 1, 2 and 3 respectively), although the analysis of the DM was relatively constant (e.g. 171, 187 and 188 g CP per kg DM, and predicted energy contents of 11.4, 11.5 and 11.5 MJ ME per kg DM). No differences in concentrate composition were seen between samples from the different experimental periods, which is as

expected since concentrates were produced in a single batch.

The mean daily intakes of silage DM, total food DM, CP, ME, estimated FME and acid hydrolysis ether extract (AHEE) are given in Table 4. Food FME concentration was estimated from the ME and AHEE contents of the concentrate (concentrate FME = ME - 0.033 × AHEE) and silage ME (silage FME = 0.71 × ME), assuming additivity (Agricultural and Food Research Council (AFRC, 1992)). Because of differences in the FME densities of the concentrate portions of the diet, the ratios of effective rumen degradable protein (ERDP) to FME of the diets consumed differed between treatments (14.3, 12.5 and 12.6 g ERDP per MJ FME).

There was a significant increase in the DM intake of animals offered the LEHF concentrate due to an increase in the silage intake. Whole tract apparent digestibility of dietary organic matter (Table 4) was calculated from the change in concentration of indigestible acid-detergent fibre between food and faeces; diet digestibility was significantly lower in animals offered the LEHF concentrate ( $P < 0.001$ ), although numerically the difference was small.

Milk production and composition were significantly affected by dietary treatment (Table 5). The concentrations of milk solids were significantly higher from animals offered the LELF concentrate. Milk yields, however, were also lowest from animals offered this concentrate. No significant differences due to treatment were seen in the ratios of protein/fat, protein/lactose or fat/lactose. Similarly, there

**Table 4** Effect of concentrate treatment on mean daily intake of dry matter (DM) and of crude protein (CP), metabolizable energy (ME) and acid hydrolysis ether extract (AHEE), and estimated intakes of effective rumen degradable protein (ERDP), digestible undegraded protein (DUP), and fermentable metabolizable energy (FME) (AFRC, 1992) (whole tract apparent organic matter (OM) digestibility and urinary purine derivative excretion expressed in relation to urinary creatinine concentration)

	Concentrate			s.e.d.	Significance†		
	LEHF (1)	LELF (2)	HEHF (3)		1-2	1-3	2-3
Silage DM (kg/day)	9.1	8.6	8.7	0.19	*	*	
Total DM (kg/day)	19.4	18.9	19.0	0.19	*	*	
CP (kg/day)	3.6	3.5	3.5				
ERDP (kg/day)	2.4	2.3	2.3				
DUP (kg/day)	0.62	0.48	0.48				
ME (MJ/day)	229	223	240				
FME (MJ/day)	168	184	182				
AHEE (kg/day)	1.5	0.8	1.4				
Whole tract apparent digestibility of OM (g/g)	0.79	0.81	0.82	0.003	***	***	
Allantoin + uric acid/creatinine (mol/mol)	3.24	3.51	3.51	0.104	*	*	

† Significance of difference of effects between concentrate treatments; 1-2 signifies difference between treatments LEHF and LELF, etc.

**Table 5** *Effect of dietary treatment on milk yield and composition, and on yields of milk components*

	Concentrate			s.e.d.	Significance†		
	LEHF (1)	LELF (2)	HEHF (3)		1-2	1-3	2-3
Milk yield (kg/day)	32.8	31.0	33.8	0.63	*		***
Crude protein (g/kg)	30.4	31.5	30.0	0.37	**		**
True protein (g/kg)	28.6	29.8	28.2	0.39	**		**
Casein (g/kg)	23.4	24.3	23.0	0.30	**		***
Whey‡ (g/kg)	5.21	5.45	5.18	0.153			
Non-urea NPN (g/kg)	0.086	0.062	0.070	0.0119			
Urea (g/kg)	0.415	0.441	0.447	0.0194			
Fat (g/kg)	32.5	34.4	31.9	1.07			*
Lactose (g/kg)	48.3	49.0	47.6	0.30	*		***
Citric acid (g/kg)	1.023	0.842	1.040	0.0189	***		***
Crude protein yield (g/day)	990	971	1005	16.5			
True protein yield (g/day)	931	917	945	16.0			
Casein yield (g/day)	763	750	773	15.3			
Whey yield (g/day)	169	168	172	3.4			
Non-urea NPN yield (g/day)	2.8	1.9	2.2	0.41	*		
Urea yield (g/day)	13.7	13.5	15.3	0.86			
Fat yield (g/day)	1068	1064	1075	40.8			
Lactose (g/day)	1584	1517	1612	34.0			*
Citric acid yield (g/day)	33.6	26.1	35.1	0.96	***		***

† Significance of difference of effects between concentrate treatments; 1-2 signifies difference between treatments LEHF and LELF, etc.

‡ Whey calculated as true protein – casein.

was no effect of diet on casein as a proportion of true protein, with a grand mean of 0.82. The concentration and yield of citric acid in milk (Table 5) increased significantly in response to additional dietary fat (LEHF *v.* LELF concentrates).

Milk concentrations of potassium, sodium, chlorine, calcium and phosphorus are presented in Table 6. Despite significant differences between the effects of diets LELF and HEHF on K and Na concentrations, the ratio of K to Na in milk was not affected by

**Table 6** *Effect of dietary treatment on mean milk mineral concentrations (values in g/kg)*

	Concentrate			s.e.d.	Significance†		
	LEHF (1)	LELF (2)	HEHF (3)		1-2	1-3	2-3
Sodium	0.40	0.39	0.41	0.011			*
Potassium	1.64	1.61	1.68	0.031			*
Chlorine	0.94	0.96	0.98	0.031			
Calcium	1.12	1.13	1.11	0.025			
Phosphorus	0.94	1.00	0.93	0.021	*		*

† Significance of difference of effects between concentrate treatments; 1-2 signifies difference between treatments LEHF and LELF, etc.

dietary treatment (means 4.2, 4.2, and 4.1 (s.e.d. 0.11) g/g, for diets LEHF, LELF, and HEHF respectively). Likewise, the lactose/Cl ratios were not significantly affected by dietary treatment (means 51.7, 51.4 and 49.2 (s.e.d. 1.86) g/g, despite significant dietary effects on milk lactose concentrations for all three diets.

The rate of excretion of the purine derivatives allantoin and uric acid, expressed in spot samples of urine as a ratio to the creatinine concentration (Table 4), was similar for animals offered the LELF and HEHF concentrates. These diets had similar dietary FME contents; animals offered the LEHF concentrate, which were supplied with approximately 15 MJ FME per day less than animals offered the other concentrates, had significantly lower rates of purine derivative excretion. The excretion of both allantoin and uric acid differed with time and day of sampling (Table 7), although the changes were in opposite directions on the 2 days of collection such that the combined purine derivative excretion data was not significantly affected by day of sampling.

The gross efficiency of dietary protein utilization for milk protein production (i.e. milk protein output/CP intake; Table 8) was significantly different between the high and low ME concentrate diets. More

**Table 7** Summary of effects of sampling time and day on purine derivative excretion: ratios of allantoin to creatinine (A/C), uric acid to creatinine (U/C), allantoin + uric acid to creatinine (AU/C), and allantoin to uric acid (A/U) (values in mol/mol)

	Time		s.e.d.	Day		s.e.d.	Significance	
	AM	PM		1	2		Time	Day
A/C	2.76	3.41	0.067	2.96	3.21	0.067	***	***
U/C	0.27	0.40	0.031	0.42	0.25	0.031	***	***
AU/C	3.03	3.81	0.081	3.38	3.46	0.081	***	
A/U	11.9	11.9	0.64	9.8	14.0	0.64		***

**Table 8** Gross efficiencies of dietary protein utilization for milk protein production (milk protein output/crude protein intake, g/g)

	Concentrate			s.e.d.	Significance†		
	LEHF (1)	LELF (2)	HEHF (3)		1-2	1-3	2-3
Crude protein	0.274	0.280	0.291	0.0040	***		*
True protein	0.257	0.264	0.273	0.0039	**		*
Casein	0.211	0.216	0.223	0.0037	**		

† Significance of difference of effects between concentrate treatments; 1-2 signifies difference between treatments LEHF and LELF, etc.

milk protein was produced per unit dietary CP consumed at the higher density of concentrate ME, particularly when comparing the two high fat concentrates.

## Discussion

Three concentrates were offered which allowed the comparison of three combinations of factors: (a) the effect of fat content at similar ME densities (LELF *v.* LEHF); (b) the effect of ME density at similar fat contents (LEHF *v.* HEHF); and (c) the effect of ME density at similar FME densities (LELF *v.* HEHF). The differences in concentrate fat and ME in (a) and (b) respectively were associated with a difference in potential FME, and the difference in ME in (c) was mediated by a difference in fat content. All three concentrates had similar CP contents, and were formulated to contain similar ratios of starch to digestible crude fibre. In practice, the concentrates contained similar levels of acid-detergent fibre (and water-soluble carbohydrates) but differed in their contents of starch, meaning that the major differences in ME supply for each concentrate were effected by their starch and fat contents.

## Food intake

Although the composition of the three concentrates differed quite markedly, as formulated, the consumption of silage was allowed to vary freely. Silage intake was highest in animals offered the LEHF concentrate, possibly as a result of its lower starch content (Thomas, 1987), although numerically the differences were small. However, because of this small increase in silage intake, animals offered the LEHF concentrate consumed more CP (and some 200 g digestible undegraded protein per day more) than animals offered the other concentrates, and yet the yields of milk protein from those animals were no different from the others.

The efficiency of use of food protein for milk protein production was significantly less from animals offered both low ME diets than those offered the high ME diet. On diets differing only in silage quality, with a constant concentrate regime, the efficiency of food protein utilization for milk production has been found to cover a considerable range (0.24 to 0.32; Dewhurst *et al.*, 1996), indicating the potential of a number of factors to influence this. However, one theory is that the utilization of amino acids for gluconeogenesis may have been reduced on the high ME diet (Lees *et al.*, 1990), leading to an increased availability for milk protein production. This would also help to explain the difference in milk protein production between the two high FME diets since both dietary CP supply and microbial protein capture were similar for these two diets.

The increase in silage intake by animals offered the LEHF concentrate was not enough to compensate for the difference between concentrate FME densities — the ERDP/FME ratio of the diet of these animals was therefore higher than that of the other diets because the ERDP intake was similar by animals on all three diets. The dietary ERDP/FME ratio is an important factor for microbial protein synthesis from rumen degradable dietary CP. For lactating dairy cows, an ERDP/FME ratio of about 11 g/MJ is recommended

(AFRC, 1992), which was exceeded by all the diets offered in this study. In this study, the urinary excretion of purine derivatives was used as a simple index of microbial protein synthesis (Moorby *et al.*, 1996). Purine derivative excretion from animals offered the two higher FME concentrates, LELF and HEHF, was significantly higher than that of animals offered the lower FME concentrate. The ERDP/FME ratio of the LEHF concentrate diet was almost 2 g/MJ higher than the other diets, indicating that the supply of FME in that diet was limiting for microbial protein production. Thus, a lack of effective nitrogen capture by the rumen microbial population may have contributed to the lower gross efficiency of milk protein production on this diet.

#### *Milk production and composition*

Milk protein concentration was significantly affected by dietary treatment and the results suggest that this was controlled by a combination of protein supply to the mammary tissue and milk volume. The daily yields of milk protein were lowest from animals offered the LELF concentrate although the large differences in milk protein concentration were brought about by a combination of protein production and milk volume. This is in agreement with many other studies in which increases in dietary fat content have decreased milk protein concentration but not decreased protein yields (e.g. Drackley and Elliot, 1993; Holter *et al.*, 1993; Palmquist *et al.*, 1993).

An increase in the supply of dietary fat led to a decrease in the concentration of milk CP. This was apparently due to significant increases in milk yields (and more specifically the volume of water produced by the animals) since there was no significant difference in the yield of milk protein between the two low ME diets. Increasing the ME supply at the high fat level did not significantly affect either milk yields or milk protein concentrations.

The effect of diet on the fatty acid content of milk fat is generally well characterized (Baer, 1991). Milk fatty acids are derived either from the blood, which in turn may be obtained from the diet (DePeters *et al.*, 1987 and 1989; Cant *et al.*, 1993), or from *de novo* synthesis by the mammary gland. Addition of palmitic acid to dairy cow diets can increase the palmitic acid content of milk fat and reduce the *de novo* synthesis of fatty acids (Banks *et al.*, 1980). The concentration of citrate in milk is an index of *de novo* fatty acid synthesis (Faulkner and Pollock, 1989) — the lower its concentration in milk, the greater the rate of fatty acid synthesis by the mammary gland — and in this study the output of citrate in milk was significantly higher on the two high fat diets than on the low fat diet, indicating a decrease in *de novo* fatty

acid synthesis. Glucose is used by the mammary gland for the production of fats — not for the incorporation of carbon but for nicotinamide adenine dinucleotide phosphate (NADP) reduction and  $\alpha$ -glycerol-P formation (Forsberg *et al.*, 1985). The incorporation of preformed fatty acids into milk fat is energetically efficient since it reduces the need for both NADPH and  $\alpha$ -glycerol-P units, because less NADPH is required for fatty acid chain elongation and because 1 g of milk fat with a high proportion of long-chain fatty acids contains fewer molecules than 1 g of fat with a greater proportion of short-chain fatty acids. If glucose is spared from the process of fatty acid synthesis, it is available for other purposes in the mammary gland. In this study, as in previous studies (Banks *et al.*, 1980; Cant *et al.*, 1993; DePeters *et al.*, 1987 and 1989), the increase in supply of dietary fatty acids apparently reduced the level of mammary fatty acid synthesis so that dietary fatty acids were incorporated into milk fat in preference to the production of new ones. At the same time as *de novo* fatty acid synthesis was decreased, milk lactose yields increased.

The daily production of milk was increased by the addition of fat to the diet, as is expected with increased lactose yields. Lactose concentrations, however, were significantly reduced by the addition of fat in the diet; this finding has also been reported by other groups (DePeters *et al.*, 1987 and 1989). The reduced lactose concentration between diets LELF and HEHF was apparently balanced by increased concentrations of potassium and sodium. The increased lactose yields on the high fat diets, and in particular on the HEHF diet suggests that more glucose was available for lactose production on these diets as less glucose was used for fatty acid synthesis.

#### *Conclusions*

In this experiment, supplementary dietary fat reduced the concentrations of milk solids. The concentration of protein, like that of fat and lactose, was reduced in the milk from animals offered high fat concentrates, despite significant increases in daily yields of protein and lactose on the high ME concentrate. The reductions in milk solids concentrations were brought about mainly through significant increases in yields of water as the *de novo* synthesis of fatty acids for milk fat was apparently reduced and lactose production was increased, drawing more water into milk and diluting the solids. Dietary fat supplied little or no FME, and this was observed in the lower rates of urinary purine derivative excretion from animals fed the low ME, high fat concentrate. Animals offered the high ME, high fat concentrate yielded more milk protein than animals offered the low ME, low fat (but equal FME)



concentrate, indicating that amino acids may have been spared from gluconeogenesis by the extra supply of ME. It is therefore concluded that the concentration of protein in milk depends not only on the supply of precursors for milk protein production, but also on the supply of precursors for fat and lactose production which will ultimately determine milk yields.

## References

- Agricultural and Food Research Council.** 1992. Technical Committee on Responses to Nutrients. Report no. 9. Nutritive requirements of ruminant animals: protein. *Nutrition Abstracts and Reviews, Series B* **62**: 788-835.
- Baer, R. J.** 1991. Alteration of the fatty acid content of milk fat. *Journal of Food Protection* **54**: 383-386.
- Banks, W., Clapperton, J. L. and Kelly, M. E.** 1980. Effect of oil-enriched diets on the milk yield and composition, and on the composition and physical properties of the milk fat of dairy cows receiving a basal ration of grass silage. *Journal of Dairy Research* **47**: 277-285.
- Cant, J. P., DePeters, E. J. and Baldwin, R. L.** 1993. Mammary uptake of energy metabolites in dairy cows fed fat and its relationship to milk protein depression. *Journal of Dairy Science* **76**: 2254-2265.
- Casper, D. P. and Schingoethe, D. J.** 1989. Model to describe and alleviate milk protein depression in early lactation dairy cows fed a high fat diet. *Journal of Dairy Science* **72**: 3327-3335.
- Coppock, C. E. and Wilks, D. L.** 1991. Supplemental fat in high-energy rations for lactating cows: effects on intake, digestion, milk yield, and composition. *Journal of Animal Science* **69**: 3826-3837.
- DePeters, E. J. and Cant, J. P.** 1992. Nutritional factors influencing the nitrogen composition of bovine milk — a review. *Journal of Dairy Science* **75**: 2043-2070.
- DePeters, E. J., Taylor, S. J. and Baldwin, R. L.** 1989. Effect of dietary fat in isocaloric rations on the nitrogen content of milk from Holstein cows. *Journal of Dairy Science* **72**: 2949-2957.
- DePeters, E. J., Taylor, S. J., Finley, C. M. and Famula, T. R.** 1987. Dietary fat and nitrogen composition of milk from lactating cows. *Journal of Dairy Science* **70**: 1192-1201.
- Dewhurst, R. J., Mitton, A. M., Offer, N. W. and Thomas, C.** 1996. Effects of the composition of grass silages on milk production and nitrogen utilization by dairy cows. *Animal Science* **62**: 25-34.
- Drackley, J. K. and Elliot, J. P.** 1993. Milk composition, ruminal characteristics, and nutrient utilization in dairy cows fed partially hydrogenated tallow. *Journal of Dairy Science* **76**: 183-196.
- Faulkner, A. and Pollock, H. T.** 1989. Changes in the concentration of metabolites in milk from cows fed on diets supplemented with soybean oil or fatty acids. *Journal of Dairy Research* **56**: 179-183.
- Forsberg, N. E., Baldwin, R. L. and Smith, N. E.** 1985. Roles of glucose and its interaction with acetate in maintenance and biosynthesis in bovine mammary tissue. *Journal of Dairy Science* **68**: 2544-2549.
- Holter, J. B., Hayes, H. H., Kierstead, N. and Whitehouse, J.** 1993. Protein-fat bypass supplement for lactating dairy cows. *Journal of Dairy Science* **76**: 1342-1352.
- Jenkins, T. C. and Jenny, B. F.** 1992. Nutrient digestion and lactation performance of dairy cows fed combinations of prilled fat and canola oil. *Journal of Dairy Science* **75**: 796-803.
- Lawes Agricultural Trust.** 1990. *Genstat 5 reference manual*. Clarendon Press, Oxford.
- Lees, J. A., Oldham, J. D., Haresign, W. and Garnsworthy, P. C.** 1990. The effect of patterns of rumen fermentation on the response by dairy cows to dietary protein concentration. *British Journal of Nutrition* **63**: 177-186.
- Moorby, J. M., Dewhurst, R. J., Thomas, C. and Marsden, S.** 1996. The influence of dietary energy source and dietary protein level on milk protein concentration from dairy cows. *Animal Science* **63**: 1-10.
- Palmquist, D. L., Weisbjerg, M. R. and Hvelplund, T.** 1993. Ruminal, intestinal and total digestibilities of nutrients in cows fed diets high in fat and undegradable protein. *Journal of Dairy Science* **76**: 1353-1364.
- Penning, P. D. and Johnson, R. H.** 1983. The use of internal markers to estimate herbage digestibility and intake. 2. Indigestible acid detergent fibre. *Journal of Agricultural Science, Cambridge* **100**: 133-138.
- Thomas, C.** 1987. Factors affecting substitution rates in dairy cows on silage based rations. In *Recent advances in animal nutrition — 1987* (ed. W. Haresign), pp. 205-218. Butterworths, London.

(Received 5 March 1997—Accepted 20 January 1998)